FORM PTO-1390 (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 514485-3880
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	U.S APPLICATION NO (If known see 37 C.F.R 1.5) 09/868824
INTERNATIONAL APPLICATION NO. PCT/EP99/10333 / INTERNATIONAL FILING DATE 22 DECEMBER 1999	PRIORITY DATE CLAIMED 23 DECEMBER 1998
TITLE OF INVENTION	**TEST SYSTEM FOR DETECTING DIFFERENT MARKERS, AND PRODUCTION AND USE THEREOF**
APPLICANT(S) FOR DO/EO/US	**Thomas WAGNER, Norbert WINDHAB**

Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 – 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:

    PCT/IB/306, 345, PCT/ISA/210
    PCT/IPEA/409, 5 sheets of drawings, 1 page Abstract

EXPRESS MAIL

Mailing Label Number: **EL742692553US**

Date of Deposit: **June 21, 2001**

I hereby certify that this paper or fee is being deposited with the United States Postal Service

"Express Mail Post Office to Addressee" Service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents and Trademarks, Box PCT Washington, DC 20231.

Charles Jacobs
(Typed or printed name of person mailing paper or fee)

Charles Jacobs
(Signature of person mailing paper or fee)

U.S. APPLICATION NO (If known, see 37 CFR 1.50)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NO.
09/868824	PCT/EP99/10333	514485-3880

21. ☒ The following fees are submitted

CALCULATIONS PTO USE ONLY

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5):
Neither international preliminary examination fee (37 CFR 1.482)
Nor international search fee (37CFR 1.445(a)(2) paid to USPTO
And International Search Report not prepared by the EPO or JPO $1000.00

International preliminary examination fee (37 C.F.R. 1.482) not paid to
USPTO but International Search Report prepared by the EPO or JPO $860.00

International prelminary examination fee (37 CFR 1.482) not paid to USPTO but
International search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)
But all claims did not satisfy provisions of PCT Article 33(1)-(4 ..... $690.00

International preliminary examination fee paid to USPTO (37 CFR 1.482)
And all claims satisfied provisions of PCT Article 33(1)-(4) $100.00

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ **860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	$	
Total Claims	**36** - 20 =	**16**	x **$18.00**	$ **288.00**	
Independent Claims	**3** - 3 =	**0**	x **$80.00**	$ **000.00**	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$ **1,148.00**	
☐ Applicant claims small entity status. See 37 C.F.R. 1.27. The fees indicated above are reduced by ½.			+	$	
SUBTOTAL =				$ **1,148.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 Months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$ **1,148.00**	
Fee for recording the enclosed assignments (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3 28, 3.31): **$40.00** per property			+	$	
TOTAL FEES ENCLOSED =				$ **1,148.00**	
				Amount to be refunded:	$
				Charged:	$

a. ☒ A check in the amount of $**1,148.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **50-0320**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. WARNING: Information on this form may become public. **Credit card information should not be included on this form**. Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

William F. Lawrence, Esq.
FROMMER LAWRENCE & HAUG LLP
745 Fifth Avenue
New York, NY 10151

SIGNATURE

William F. Lawrence, Esq.
NAME

28,029
REGISTRATION NUMBER

Date: June 21, 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	WAGNER et al.
U.S. Serial No.	:	To be assigned
Filing Date	:	Herewith
For	:	TEST SYSTEM FOR DETECTING DIFFERENT MARKS, AND PRODUCTION AND USE THEREOF

745 Fifth Avenue
New York, NY 10151

EXPRESS MAIL

Mailing Label Number: EL742692553US

Date of Deposit: June 21, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.

Charles Jackson
(Typed or printed name of person mailing paper or fee)

Charles Jackson
(Signature of person mailing paper or fee)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Box PCT
Washington, D.C. 20231

Dear Sir:

Preliminary to the examination of this U.S. national phase application, please enter the following amendments:

IN THE CLAIMS:

Please cancel Claims 1-36 and replace them with Claims 37-72:

--37. Detection process comprising the following steps:

(a) treatment of a sample comprising a first and a second marker with a first recognition species which recognizes the first marker,

(b) treatment of the sample with a second recognition species which recognizes both the first marker and the second marker,

(c) treatment of the sample with a third recognition species which recognizes the second marker,

(d) detection of the presence or absence of the first and the second marker in the sample, by the detection of the presence or absence of a complex of the recognition species and markers mentioned.

38. Detection process comprising the following steps:

(a) treatment of a sample comprising a first and a second marker with a first recognition species which recognizes the first marker,

(b) treatment of the sample with a second recognition species which recognizes the first marker and a third recognition species,

(c) treatment of the sample with a third recognition species which recognizes the second marker and the second recognition species,

(d) detection of the presence or absence of the first and the second marker in the sample, by the detection of the presence or absence of a complex of the recognition species and markers mentioned.

39. Detection process according to Claim 37 or 38, characterized in that further recognition species which recognize further markers are employed in further treatment steps.

40. Detection process according to one of Claims 37-39, characterized in that a recognition species, preferably the first recognition species, is immobilized on a support.

41. Detection process according to Claim 40, characterized in that the support is selected from a solid or gelatinous material, in particular chip material and/or thin layers of the material, preferably ceramic, metal, in particular noble metal, glasses, plastics, crystalline materials or (bio)molecular filaments, in particular cellulose or structural proteins.

42. Detection process according to one of Claims 37-41, characterized in that the recognition species and/or the marker mentioned is a synthetic substance, a natural substance and/or a natural substance derivative, preferably selected from a peptide, peptoid, protein, saccharide or a nucleic acid.

43. Detection process according to Claim 42, characterized in that the synthetic substance, a natural substance or a natural substance derivative is selected from a receptor or a functional part thereof, in particular from the extracellular domain of a membrane-based receptor, an antibody or a functional part thereof, in particular an Fv fragment, a single-chain Fv fragment (ScFv) or an Fab fragment, a cell constituent, in particular a lipid, glycoprotein, filament constituent, lectin, liposome, mitogen, antigen, secondary metabolite or hapten, a cell, in particular a lymphoid cell, or a virus, in particular a virus constituent, especially a capsid, or a viroid, or a derivative, in particular an acetate, or their active parts, or a single-stranded or double-stranded nucleic acid, in particular a natural nucleic acid in the form of a DNA or RNA or an unnatural nucleic acid, preferably p-RNA, p-DNA, PNA or CNA, or hybrids of the substances mentioned.

44. Detection process according to one of Claims 37-43, characterized in that the recognition of a marker by a recognition species takes place by means of non-covalent interactions, in particular by means of hydrogen bonds, salt bridges, stacking, formation of metal ligands, charge-transfer complexes, Van-der-Waals forces or hydrophobic interactions.

45. Detection process according to one of Claims 37-44, characterized in that at least one recognition species is labelled, in particular all recognition species are labelled, preferably at least two recognition species are differently labelled.

46. Detection process according to Claim 45, characterized in that the marker is a non-radioactive marker or radioactive marker, preferably an LOCI marker, FRET marker, fluorescence quenching marker, SPA marker, fluorescence marker, enzymatic marker, redox marker or spin marker.

47. Detection process according to one of Claims 37-46, characterized in that the marker and/or the signal is amplified.

48. Detection process according to one of Claims 37-47, characterized in that the detection is carried out competitively according to step (d) of the process.

49. Detection process according to one of Claims 37-48, characterized in that at least one marker is a natural or unnatural, single-stranded or double-stranded nucleic acid and each further marker is a synthetic substance, a different natural substance or a different natural substance derivative other than a nucleic acid, preferably an antigen.

50. Detection process according to one of Claims 37-48, characterized in that the first marker and each further marker is a natural or unnatural, single-stranded or double-stranded nucleic acid or alternatively a synthetic substance, a different natural substance or a different natural substance derivative other than a natural nucleic acid, preferably an antigen.

51. Detection process according to one of Claims 37-50, characterized in that a natural or unnatural, single-stranded or double-stranded nucleic acid as a marker is recognized by a natural or unnatural, single-stranded or double-stranded nucleic acid as recognition species.

52. Detection process according to one of Claims 37-51, characterized in that a synthetic substance, a natural substance or a natural substance derivative is recognized by a synthetic substance, a natural substance or a natural substance derivative, preferably by an antibody or an antibody derivative, as recognition species.

53. Detection process according to one of Claims 37-52, characterized in that at least one recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid and each further recognition species is a synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or an antibody derivative.

54. Detection process according to one of Claims 37-52, characterized in that the first recognition species and each further recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid or alternatively a synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or an antibody derivative.

55. Detection process according to one of Claims 37-52, characterized in that at least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and another natural or unnatural, single-stranded or double-stranded nucleic acid.

56. Detection process according to one of Claims 37-52, characterized in that at least one recognition species is a hybrid of a synthetic substance, a natural substance or a natural substance derivative and another synthetic substance, another natural substance or another natural substance derivative.

57. Detection process according to one of Claims 37-52, characterized in that at least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and a synthetic substance, a different natural substance or a different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

58. Detection process according to one of Claims 37-52, characterized in that a first recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid, a second recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and a synthetic substance, a natural substance or a natural substance derivative, preferably an antibody or antibody derivative.

59. Detection process according to one of Claims 37-52, characterized in that a first recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid, a second recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and another natural or unnatural, single-stranded or double-stranded nucleic acid, and the third recognition species is a further different natural or unnatural, single-stranded or double-stranded nucleic acid.

60. Detection process according to one of Claims 37-52, characterized in that a first recognition species is a synthetic substance, a natural substance or a natural substance derivative, preferably an antibody or antibody derivative, a second recognition species is a hybrid of a synthetic substance, a natural substance or a natural substance derivative, preferably an antibody or antibody derivative, and another natural substance or another natural substance derivative, preferably another antibody or antibody derivative, and a third recognition species is a further different synthetic substance, a natural substance or a natural substance derivative, preferably a further different antibody or antibody derivative.

61. Test system for the detection of the presence or absence of at least two different markers in a sample comprising at least two recognition species which recognize at least two different markers with formation of a complex, at least two of the recognition species being differently labelled.

62. Test system according to Claim 61, characterized in that at least one recognition species is immobilized on a support.

63. Test system according to Claim 61 or 62, characterized in that at least one recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid and at least one other recognition species is another natural or unnatural, single-stranded or double-stranded nucleic acid.

64. Test system according to Claim 61 or 62, characterized in that at least one recognition species is a synthetic substance, a different natural substance or a different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative, and at least one other recognition species is a synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

65. Test system according to Claim 61 or 62, characterized in that at least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and a synthetic substance, a different natural substance or a different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

66. Test system according to Claim 61 or 62, characterized in that at least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and another natural or unnatural, single-stranded or double-stranded nucleic acid.

67. Test system according to Claim 61 or 62, characterized in that at least one recognition species is a hybrid of a synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative, and another synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

68. Process for the production of a test system according to one of Claims 61-67, characterized in that the individual recognition species are assembled.

69. Process according to Claim 68, characterized in that at least one recognition species is immobilized on a support.

70. Use of the test system according to one of Claims 61-67 for the detection of the presence and/or absence of at least two different markers in a sample.

71. Use of the test system according to Claim 68 in the form of a diagnostic or in the form of an analyte.

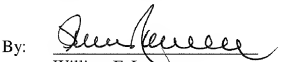
72. Use of the test system according to Claim 68 or 69 for the detection of a disorder or for environmental analysis, in particular for the detection of disease pathogens, markers of diseases, toxins and/or allergens.--

REMARKS

The claims have been amended to include amendments that were made during International Preliminary Examination. No new matter has been added.

Entry of this amendment and an early examination on the merits are respectfully solicited.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP

By: 

William F. Lawrence
Reg. No. 28,029
(212) 588-0800

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s)	:	WAGNER et al.
U.S. Serial No.	:	09/868,824
Filing Date	:	June 21, 2001
For	:	TEST SYSTEM FOR DETECTING DIFFERENT MARKERS, AND PRODUCTION AND USE THEREOF

745 Fifth Avenue, New York, NY 10151

EXPRESS MAIL

Mailing Label Number: EL 819168702 US

Date of Deposit: August 1, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.

(Typed or printed name of person mailing paper or fee)

(Signature of person mailing paper or fee)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

This is in response to the Notice to Comply With Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence mailed on July 18, 2001. Applicants respectfully request acceptance of the enclosed paper copy and computer readable form of the Sequence Listing. It is also respectfully requested that the application be amended as follows:

IN THE SPECIFICATION:

Please replace Table1 beginning at page 12, line 1, with the following rewritten table:

--

Reagent	Specification
DNA 1 (recognition species 1)	Texas Red-5'-AAA-TGC-ATG-TCG-TCG-TGA-TGT-AAA-3' (SEQ ID NO: 1)
DNA 2 (marker 1)	5'-ATT-GTC-ATA-ATC-ATC-TTG-AGA-CGC-TTT-TTT-TTT-TTT-ACA-TCA-CGA-CGA-CAT-GCA-TTT-3' (SEQ ID NO: 2)
DNA 3 (recognition species 2)	Biotin-5'-GCG-TCT-CAA-CAT-GAT-TAT-GAC-AAT-3' (SEQ ID NO: 3)
Antibodies (recognition species 3	Streptavidin-conjugated anti-human IgG F(ab')$_2$ (goat)
Antigen (marker 2)	Fluorescein-labelled human IgG F(ab')$_2$ fragment

Table 1: Recognition species and markers used.--;

Please replace Table2 beginning at page 13, line 18, with the following rewritten table:

--

Reagent	Specification
DNA 1' (recognition species 1)	Biotin-5'-AAA-TGC-ATG-TCG-TCG-TGA-TGT-AAA-3' (SEQ ID NO: 4)
DNA 2 (marker 1)	5'-ATT-GTC-ATA-ATC-ATC-TTG-AGA-CGC-TTT-TTT-TTT-TTT-ACA-TCA-CGA-CGA-CAT-GCA-TTT-3' (SEQ ID NO: 2)
DNA 3 (recognition species 2)	Biotin-5'-GCG-TCT-CAA-CAT-GAT-TAT-GAC-AAT-3' (SEQ ID NO: 3)
Antibodies (recognition species 3	Streptavidin-conjugated anti-human IgG F(ab')$_2$ (goat)
Antigen (marker 2)	Fluorescein-labelled human IgG F(ab')$_2$ fragment

Table 2: Recognition species and markers used.--;

Immediately after page 14 and before the first page of claims (page 15), if appropriate, please insert the enclosed pages identified as --Sequence Listing--. Please renumber the pages accordingly.

REMARKS

A paper-copy of the nucleotide sequence listing and a computer readable form (floppy disk) of the nucleotide sequence Listing are enclosed.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

These amendments are introduced merely to assign the correct SEQ ID NO: and to place the nucleotide sequence listing in the application, (after the specification and before the claims). It is respectfully asserted that these amendments do not add any new matter.

In view of the amendments, remarks and enclosures, the application complies with the requirements for computer readable disclosure of the biological sequences under 37 C.F.R. §1.821-1.825.

If any additional fees are incurred for entry and consideration of this Amendment, the Examiner is authorized to charge any fees or credit any overpayment to Deposit Account No. 50-0320.

Respectfully submitted,
FROMMER LAWRENCE & HAUG LLP

By: 

Susan K. Lehnhardt
Reg. No. 33,943
(212) 588-0800

Version with markings to show changes made.

IN THE SPECIFICATION:

Table1 beginning at page 12, line 1,has been amended as shown:

Reagent	Specification
DNA 1 (recognition species 1)	Texas Red-5'-AAA-TGC-ATG-TCG-TCG-TGA-TGT-AAA-3' (SEQ ID NO: 1)
DNA 2 (marker 1)	5'-ATT-GTC-ATA-ATC-ATC-TTG-AGA-CGC-TTT-TTT-TTT-TTT-ACA-TCA-CGA-CGA-CAT-GCA-TTT-3' (SEQ ID NO: 2)
DNA 3 (recognition species 2)	Biotin-5'-GCG-TCT-CAA-CAT-GAT-TAT-GAC-AAT-3' (SEQ ID NO: 3)
Antibodies (recognition species 3	Streptavidin-conjugated anti-human IgG $F(ab')_2$ (goat)
Antigen (marker 2)	Fluorescein-labelled human IgG $F(ab')_2$ fragment

Table 1: Recognition species and markers used.

Table 2 beginning at page 13, line 18, has been amended as shown:

Reagent	Specification
DNA 1' (recognition species 1)	Biotin-5'-AAA-TGC-ATG-TCG-TCG-TGA-TGT-AAA-3' (SEQ ID NO: 4)
DNA 2 (marker 1)	5'-ATT-GTC-ATA-ATC-ATC-TTG-AGA-CGC-TTT-TTT-TTT-TTT-ACA-TCA-CGA-CGA-CAT-GCA-TTT-3' (SEQ ID NO: 2)
DNA 3 (recognition species 2)	Biotin-5'-GCG-TCT-CAA-CAT-GAT-TAT-GAC-AAT-3' (SEQ ID NO: 3)
Antibodies (recognition species 3	Streptavidin-conjugated anti-human IgG $F(ab')_2$ (goat)
Antigen (marker 2)	Fluorescein-labelled human IgG $F(ab')_2$ fragment

Table 2: Recognition species and markers used.

Test system for the recognition of different markers, its preparation and use

The present invention relates to a test system comprising at least two recognition species which recognize at least two different markers with formation of a complex, its preparation and use in a suitable detection process.

The areas of application of test systems, such as diagnostics, are widespread in biology, biochemistry, medicine and pharmacology. Especially in medicine, a reliable and clear diagnosis of diseases, such as viral infections or cancer, is of extreme importance for increasing the quality of life, since only by early recognition of a disease can a timely and effective treatment take place. Based on the recognition of disease-specific markers or ligands, such as nucleic acid sequences, proteins or antigens, the pathogen or the disease in the biological sample is detected. Diagnostic tests are widespread in which a marker or a class of markers in each case is detected, such as in ELISA or in amplification methods, such as PCR, b-DNA, Southern, Western or Northern blotting. The types of detection used range from simple staining methods and calorimetric methods via fluorescence energy transfer (FRET) and fluorescence quenching up to scintillation proximity assay (SPA).

A significant disadvantage in the use of only one marker or one class of marker is that false-positive test results easily result, which also lead to wrong conclusions regarding a specific disease. A second test or still further tests must often therefore be carried out on the same or complementary analytes in order to be able to make a reliable statement with regard to sickness/health. This leads to more tests, whose results are to be compared with one another, which is at the same time laborious and cost-intensive.

It is therefore an object of the present invention to develop qualitatively better, less complicated and less expensive analyte tests than those already known.

Surprisingly, it has now been found that the linkage of two or more test results at the molecular level in the sense of Boolean linkage allows a qualitatively very good, simple and inexpensive analyte test, the different test results essentially not interfering with one another.

The invention therefore relates to a detection process comprising the following steps:

(a) treatment of a sample comprising a first and a second marker with a first recognition species which recognizes the first marker,

(b) treatment of the sample with a second recognition species which recognizes both the first marker and the second marker,

(c) treatment of the sample with a third recognition species which recognizes the second marker,

(d) detection of the presence or absence of a complex of the recognition species and markers mentioned.

In addition, the present invention relates to a detection process comprising the following steps:

(a) treatment of a sample comprising a first and a second marker with a first recognition species which recognizes the first marker,

(b) treatment of the sample with a second recognition species which recognizes the first marker and a third recognition species,

(c) treatment of the sample with a third recognition species which recognizes the second marker and the second recognition species,

(d) detection of the presence or absence of a complex of the recognition species and markers mentioned.

To increase the specificity, it is advantageous in a further embodiment that further recognition species which recognize further markers are employed in further treatment steps, which results in n + 1 recognition species on widening to n ligands where n is equal to a natural number.

In further preferred embodiments, the detection process is carried out in homogenous, partly homogeneous (modular) or immobilized form. In a homogeneous embodiment, the binding events are produced stepwise and the complex possibly formed is detected in a "proximity assay". The first and the last components are preferably labelled such that they can only produce a signal when mutually opposite. Preferred detection processes are, for example, LOCI (Ullmann, E. et al. (1994) Proc. Natl. Acad. Sci. USA 91, 5426), fluorescence energy transfer (FRET) Cardullo, R.A. (1992) in "Nonradioactive Labeling and Detection of Biomolecules", 414-423, Springer Verlag), fluorescence quenching (Ladokhin, A.S. (1997) "Distribution analysis of depth-dependent fluorescence quenching in membranes: a practical guide", Methods Enzymol., 278, 462-473) or scintillation proximity assay (SPA) (Picardo, M. & Hughes, K.T. (1997) "Scintillation proximity assays. High Throughput Screening", Ed. Devlin, J.P.; Verlag Dekker, New York, N.Y., 307-316).

In a partly homogeneous or modular embodiment, the binding events are produced stepwise and in solution and, as soon as the complex has formed, it is bound to a solid support via one of the components. The complex formed is detected by means of a marker, in particular a non-radioactive marker or radioactive marker, preferably by means of a fluorescence marker, enzymatic marker, redox marker or spin marker (Kessler, C. (Ed.) Nonradioactive Labeling and Detection of Biomolecules (1992), Springer Verlag, 414-423).

In an immobilized embodiment, a recognition species, preferably the first recognition species, is bound to a solid support and subsequently built up by

stepwise addition of the other components of the complex. Labelling is preferably carried out by methods which are identical or similar to those in the case of the partly homogeneous embodiment.

A suitable support for the immobilization is especially solid or gelatinous material, in particular chip material and/or thin layers of the material, preferably ceramic, metal, in particular noble metal, glasses, plastics, crystalline materials or (bio)-molecular filaments, in particular cellulose or structural proteins.

The recognition species and/or markers employed in the detection process according to the invention are, in particular, a synthetic substance, a natural substance and/or a natural substance derivative, preferably a peptide, peptoid, protein, saccharide or a nucleic acid. A receptor or a functional part thereof, for example, is particularly preferred, in particular a functional part which originates from the extracellular domain of a membrane-based receptor, an antibody or a functional part thereof, in particular an Fv fragment (Skerra & Plückthun (1988), Science 240, 1038), a single-chain Fv fragment (scFv; Bird et al. (1988), Science 242, 423; Huston et al. (1988), Proc. Natl. Acad. Sci. U.S.A. 85, 5879) or an Fab fragment (Better et al. (1988), Science 240, 1041), an aptamer, for example a DNA or RNA aptamer or derivatives thereof, for example aptamers provided with protective groups customary in nucleic acid chemistry, a cell constituent, in particular a lipid, glycoprotein, filament constituent, lectin, liposome, mitogen, antigen, secondary metabolite or hapten, a cell, in particular a lymphoid cell, or a virus, in particular a virus constituent, especially a capsid, or a viroid or a derivative, in particular an acetate, or their active parts, or a single-stranded or double-stranded nucleic acid, in particular DNA, RNA, p-RNA (Pitsch, S. et al., Helv. Chim. Acta. (1993), 76, 2161; Pitsch, S. et al., Helv. Chim. Acta, (1995), 78, 1621), p-DNA

(DE 198 37 387.2), PNA (peptide nucleic acid; Nielsen, P.E. et al. (1991) Science, 254, 1497), CNA (Aminocyclohexylnucleic acid; PCT/EP98/06002) or an aptamer (see, for example, Bock, L.C. et al. (1992) Nature, 355, 564) or hybrids of the substances mentioned.

According to the present invention, aptamers, on account of their binding properties to specific molecules which are different from nucleic acids, such as proteins, do not belong to the nucleic acids, but to antibody derivatives. DNA aptamers or RNA aptamers are preferred.

The nucleic acids according to the invention including aptamers can also be modified. For this, the methods known from nucleic acid chemistry to the person skilled in the art can be used. Modifications are preferred which lead to stabilization of the nucleic acids (see, for example, Ullmann, E. & Peyman, A. (1990) Chemical Reviews, 90, 543, No. 4).

Customarily, the recognition of a marker by a recognition species takes place by means of non-covalent interactions, in particular by means of hydrogen bonds, salt bridges, stacking, formation of metal ligands, charge-transfer complexes, Van-der-Waals forces or hydrophobic interactions. For example, a nucleic acid is recognized as a marker by a completely or partly complementary nucleic acid or a synthetic substance, such as a chemical, a natural substance and/or a natural substance derivative are recognized as antigenic substances by an appropriate antibody or antibody derivative. According to the detection process according to the invention, the markers can belong to any desired class of substance, but preferably of at least two different classes.

According to the detection process according to the invention, additionally at least one recognition species is labelled, preferably all recognition species are labelled, especially at least two recognition species are differently labelled. As already

illustrated above in greater detail, the marker, depending on whether a homogeneous, partly homogeneous (modular) or immobilized embodiment is concerned, can be non-radioactive or radioactive, preferably LOCI, FRET, fluorescence quenching, SPA, a fluorescence marker, enzymatic marker, redox marker or spin marker.

In a further preferred embodiment, the marker and/or the signal can be amplified, which leads to an increase in the sensitivity of the detection process. The amplification of the marker relates, in particular, to the amplification of nucleic acids, for example by PCR, NASBA, LCR, SDA, Qβ replication or RT-PCR (Kessler, C. (1992) supra). The signal amplification is achieved, for example, by 'cross-linking' of binding components, antibody or nucleic acid trees (e.g. b-DNA), catalytic substrate reaction (e.g. alkaline phosphatase, peroxidase, β-galactosidase) or signal cascades.

In addition to the mentioned in-vitro amplification, in-vivo amplification is also possible, e.g. detection of r-RNA, indirect detection of antigens.

In principle, the markers can be divided into two classes. In the case of 'positive markers', the absence of these markers is detected, for example, by means of the absence of a signal. Positive markers refer in general to markers present in a healthy organism, e.g. m-RNA. Negative markers are in general designated as the substances of a pathogen or of an ill organism, which can be determined by means of the detection process according to the invention .

In the detection process according to the invention, either two or more negative, two or more positive or two or more positive and negative markers can be detected. The detection thus takes place either via the occurrence or via the absence of a signal. Likewise, a displacement of a signal, e.g. by the displacement of a molecule from a complex or from its binding conformation (e.g. Molecular Beacons, S. Tyaki,

Kramer F.R., Nature Biotechnology 14, 303-308, 1996; R.P. Ekins, Clinical Chemistry, 44/9, 2015-2030, 1998) is possible in a competitive assay. For this, a substance is added to the test system which displaces one of the markers to be detected, the molecular complex built up from markers and recognition species and thus also the signal associated therewith disappearing. By means of a titration, the concentration of the displaced marker can thus be determined in a simple manner.

The detection process according to the invention can now be present in at least one of the following alternative embodiments, which are particularly preferred:

1. At least one marker is a natural or unnatural, single-stranded or double-stranded nucleic acid and each further marker is a synthetic substance, natural substance or natural substance derivative other than a nucleic acid, preferably an antigen.
2. The first marker and each further marker is a natural or unnatural, single-stranded or double-stranded nucleic acid or alternatively a synthetic substance, a natural substance or a natural substance derivative other than a nucleic acid, preferably an antigen.
3. A natural or unnatural, single-stranded or double-stranded nucleic acid as a marker is recognized by a natural or unnatural, single-stranded or double-stranded nucleic acid as recognition species.
4. A synthetic substance, a natural substance or a natural substance derivative is recognized by a synthetic substance, a natural substance or a natural substance derivative, preferably by an antibody or an antibody derivative, as recognition species.
5. At least one recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid and each further recognition species is a synthetic substance, different natural

substance or different natural substance derivative other than a nucleic acid, preferably an antibody or an antibody derivative.

6. The first recognition species and each further recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid or alternatively a synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or an antibody derivative.

7. At least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and another natural or unnatural, single-stranded or double-stranded nucleic acid.

8. At least one recognition species is a hybrid of a synthetic substance, a natural substance or a natural substance derivative and another synthetic substance, another natural substance or another natural substance derivative.

9. At least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and a synthetic substance, a different natural substance or a different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

10. A first recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid, a second recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and a synthetic substance, a natural substance or a natural substance derivative, preferably an antibody or antibody derivative.

11. A first recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid, a second recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and another natural

or unnatural single-stranded or double-stranded nucleic acid, and the third recognition species is a further different natural or unnatural, single-stranded or double-stranded nucleic acid.

12. A first recognition species is a synthetic substance, a natural substance or a natural substance derivative, preferably an antibody or antibody derivative, a second recognition species is a hybrid of a synthetic substance, a natural substance or a natural substance derivative, preferably an antibody or antibody derivative, and another synthetic substance, another natural substance or another natural substance derivative, preferably another antibody or antibody derivative, and a third recognition species is a further different synthetic substance, a further different natural substance or a further different natural substance derivative, preferably a further different antibody or a further different antibody derivative.

Another subject of the present invention is a test system comprising at least two recognition species, which recognize at least two different markers with formation of a complex, preferably the recognition species or markers already described above. In a preferred embodiment, at least one recognition species is immobilized on a support, such as preferably already described above in greater detail.

The test system according to the invention can be employed in the following preferred embodiments:

1. At least one recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid and at least one other recognition species is another natural or unnatural, single-stranded or double-stranded nucleic acid.
2. At least one recognition species is a synthetic substance, a different natural substance or a different natural substance derivative other than a nucleic acid, preferably an antibody or antibody

derivative, and at least one other recognition species is another synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

3. At least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and a synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

4. At least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and another natural or unnatural single-stranded or double-stranded nucleic acid.

5. At least one recognition species is a hybrid of a synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative, and another synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

The test system according to the invention can be produced, for example, by assembling the recognition species necessary for the individual embodiments, or by immobilizing at least one recognition species on a support, such as preferably already described above, by the process generally known to the person skilled in the art.

The test system according to the invention can be employed in the detection process according to the invention, as described in greater detail above. In particular, it is used for the detection of the presence and/or absence of at least two different markers in a sample. It is preferably present in the form of a diagnostic or an analyte. It is therefore used, in particular, for the detection of disorders or

for environmental analysis, in particular for the detection of toxins and/or allergens.

The following figures and examples are intended to describe the invention in greater detail, without restricting it.

DESCRIPTION OF THE FIGURES

Fig. 1 shows schematically the detection of two analytes (A and B) in an assay in the immobilized embodiment.

Fig. 2 shows schematically the detection of two analytes (antigens A and B) in an assay in the immobilized embodiment.

Fig. 3 shows schematically the detection of two analytes (nucleic acid A and B) in an assay in the immobilized embodiment.

Fig. 4 shows schematically the complex of markers and recognition species according to Example 1.

Fig. 5 shows schematically the complex of markers and recognition species according to Example 2.

EXAMPLES

Simultaneous detection of a deoxyribonucleic acid and of a labelled **antibody/antigen**

Starting compounds:

The reagents needed for the example, such as Texas Red®-labelled oligonucleotide conjugate (24-mer DNA; Interactiva; DNA 1), a biotinylated oligonucleotide conjugate (24-mer DNA; Interactiva; DNA 3); a synthetic oligonucleotide (57-mer DNA; Interactiva; DNA 2), which has sequences complementary to the two other DNAs, streptavidin-conjugated anti-human IgG $F(ab')_2$ (goat; Rockland) and a fluorescein-labelled human IgG $F(ab')_2$ fragment (Rockland) as antigen, are all commercially obtainable

Reagent	Specification
DNA 1 (recognition species 1)	Texas Red-5'-AAA-TGC-ATG-TCG-TCG-TGA-TGT-AAA-3'
DNA 2 (marker 1)	5'-ATT-GTC-ATA-ATC-ATC-TTG-AGA-CGC-TTT-TTT-TTT-TTT-ACA-TCA-CGA-CGA-CAT-GCA-TTT-3'
DNA 3 (recognition species 2)	Biotin-5'-GCG-TCT-CAA-CAT-GAT-TAT-GAC-AAT-3'
Antibodies (recognition species 3	Streptavidin-conjugated anti-human IgG $F(ab')_2$ (goat)
Antigen (marker 2)	Fluorescein-labelled human IgG $F(ab')_2$ fragment

Table 1: Recognition species and markers used.

Example 1

1 nmol of Texas Red-labelled oligonucleotide conjugate (DNA 1), 1 nmol of biotin-labelled oligonucleotide conjugate (DNA 3) and one pmol of the 57-mer oligonucleotide (DNA 2) were taken up in 150 µl of hybridization buffer (5 × SSC, 0.02% SDS) in each case. The constituents were heated at 60°C for 30 min, mixed with one another and incubated at 37°C for 3 h. They were allowed to cool to room temperature (RT), 1 nmol of the streptavidin anti-human IgG $F(ab')_2$ conjugate and 1 nmol of the fluorescein-labelled IgG $F(ab')_2$ fragment were added and the mixture was allowed to stand at RT overnight. The complex formed was detected by means of a yellow band in a non-denaturing gel (15% strength TEB gel, BioRad).

Example 2

1 nmol of biotin-labelled oligonucleotide conjugate (DNA 1), 1 nmol of biotin-labelled oligonucleotide conjugate (DNA 3) and one pmol of the 57-mer oligonucleotide (DNA 2) were taken up in 150 µl of hybridization buffer (5 × SSC, 0.02% SDS) in each

case. The constituents were heated at 60°C for 5 min, mixed with one another and incubated at 37°C for 3 h. The solution was allowed to cool to room temperature (RT) and added to a streptavidin-coated microtitre plate (BIOTEZ, order No. 040298920). The supernatant solution was removed by pipette and the support was washed 5× with 500 µl of 0.9% NaCl solution. 200 µl of a solution of 200 µl of streptavidin anti-human IgG $F(ab')_2$ (goat) solution (1.6 mg/ml) preincubated at RT for 2 h and 40 µl of the fluorescein-labelled IgG $F(ab')_2$ fragment solution (5.0 mg/ml) were then added and the mixture was incubated at RT for 1-2 h. The supernatant solution was in turn removed by pipette and the support was washed 5× with 500 µl of 0.9% NaCl solution. The formation of the complex was detected by measuring the fluorescence of the fluorescein ($\lambda_{max,A}$: 494 nm, $\lambda_{max,E}$: 525 nm).

Reagent	**Specification**
DNA 1' (recognition species 1)	Biotin-5'-AAA-TGC-ATG-TCG-TCG-TGA-TGT-AAA-3'
DNA 2 (marker 1)	5'-ATT-GTC-ATA-ATC-ATC-TTG-AGA-CGC-TTT-TTT-TTT-TTT-ACA-TCA-CGA-CGA-CAT-GCA-TTT-3'
DNA 3 (recognition species 2)	Biotin-5'-GCG-TCT-CAA-CAT-GAT-TAT-GAC-AAT-3'
Antibodies (recognition species 3	Streptavidin-conjugated anti-human IgG $F(ab')_2$ (goat)
Antigen (marker 2)	Fluorescein-labelled human IgG $F(ab')_2$ fragment

Table 2: Recognition species and markers used.

It will be apparent to those skilled in the art that various modifications and variations can be made to the compositions and processes of this invention. Thus, it is intended that the present invention cover such

modifications and variations, provided they come within the scope of the appended claims and their equivalents.

Priority application DE 198 59 912.9, filed December 23, 1998, including the specification, drawings, claims and abstract, is hereby incorporated by reference. All publications cited herein are incorporated in their entireties by reference.

Patent Claims

1. Detection process comprising the following steps:

(a) treatment of a sample comprising a first and a second marker with a first recognition species which recognizes the first marker,

(b) treatment of the sample with a second recognition species which recognizes both the first marker and the second marker,

(c) treatment of the sample with a third recognition species which recognizes the second marker,

(d) detection of the presence or absence of a complex of the recognition species and markers mentioned.

2. Detection process comprising the following steps:

(a) treatment of a sample comprising a first and a second marker with a first recognition species which recognizes the first marker,

(b) treatment of the sample with a second recognition species which recognizes the first marker and a third recognition species,

(c) treatment of the sample with a third recognition species which recognizes the second marker and the second recognition species,

(d) detection of the presence or absence of a complex of the recognition species and markers mentioned.

3. Detection process according to Claim 1 or 2, characterized in that further recognition species which recognize further markers are employed in further treatment steps.

4. Detection process according to one of Claims 1-3, characterized in that a recognition species,

preferably the first recognition species, is immobilized on a support.

5. Detection process according to Claim 4, characterized in that the support is selected from a solid or gelatinous material, in particular chip material and/or thin layers of the material, preferably ceramic, metal, in particular noble metal, glasses, plastics, crystalline materials or (bio)molecular filaments, in particular cellulose or structural proteins.

6. Detection process according to one of Claims 1-5, characterized in that the recognition species and/or the marker mentioned is a synthetic substance, a natural substance and/or a natural substance derivative, preferably selected from a peptide, peptoid, protein, saccharide or a nucleic acid.

7. Detection process according to Claim 6, characterized in that the synthetic substance, a natural substance or a natural substance derivative is selected from a receptor or a functional part thereof, in particular from the extracellular domain of a membrane-based receptor, an antibody or a functional part thereof, in particular an Fv fragment, a single-chain Fv fragment (ScFv) or an Fab fragment, a cell constituent, in particular a lipid, glycoprotein, filament constituent, lectin, liposome, mitogen, antigen, secondary metabolite or hapten, a cell, in particular a lymphoid cell, or a virus, in particular a virus constituent, especially a capsid, or a viroid, or a derivative, in particular an acetate, or their active parts, or a single-stranded or double-stranded nucleic acid, in particular a natural nucleic acid in the form of a DNA or RNA or an unnatural nucleic acid, preferably p-RNA, p-DNA, PNA or CNA, or hybrids of the substances mentioned.

8. Detection process according to one of Claims 1-7, characterized in that the recognition of a marker by a recognition species takes place by means of non-covalent interactions, in particular by means of

hydrogen bonds, salt bridges, stacking, formation of metal ligands, charge-transfer complexes, Van-der-Waals forces or hydrophobic interactions.

9. Detection process according to one of Claims 1-8, characterized in that at least one recognition species is labelled, in particular all recognition species are labelled, preferably at least two recognition species are differently labelled.

10. Detection process according to Claim 9, characterized in that the marker is a non-radioactive marker or radioactive marker, preferably an LOCI marker, FRET marker, fluorescence quenching marker, SPA marker, fluorescence marker, enzymatic marker, redox marker or spin marker.

11. Detection process according to one of Claims 1-10, characterized in that the marker and/or the signal is amplified.

12. Detection process according to one of Claims 1-11, characterized in that the detection is carried out competitively according to step (d) of the process.

13. Detection process according to one of Claims 1-12, characterized in that at least one marker is a natural or unnatural, single-stranded or double-stranded nucleic acid and each further marker is a synthetic substance, a different natural substance or a different natural substance derivative other than a nucleic acid, preferably an antigen.

14. Detection process according to one of Claims 1-12, characterized in that the first marker and each further marker is a natural or unnatural, single-stranded or double-stranded nucleic acid or alternatively a synthetic substance, a different natural substance or a different natural substance derivative other than a natural nucleic acid, preferably an antigen.

15. Detection process according to one of Claims 1-14, characterized in that a natural or unnatural, single-stranded or double-stranded nucleic acid as a marker is recognized by a natural or unnatural, single-

stranded or double-stranded nucleic acid as recognition species.

16. Detection process according to one of Claims 1-15, characterized in that a synthetic substance, a natural substance or a natural substance derivative is recognized by a synthetic substance, a natural substance or a natural substance derivative, preferably by an antibody or an antibody derivative, as recognition species.

17. Detection process according to one of Claims 1-16, characterized in that at least one recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid and each further recognition species is a synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or an antibody derivative.

18. Detection process according to one of Claims 1-16, characterized in that the first recognition species and each further recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid or alternatively a synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or an antibody derivative.

19. Detection process according to one of Claims 1-16, characterized in that at least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and another natural or unnatural, single-stranded or double-stranded nucleic acid.

20. Detection process according to one of Claims 1-16, characterized in that at least one recognition species is a hybrid of a synthetic substance, a natural substance or a natural substance derivative and another synthetic substance, another natural substance or another natural substance derivative.

21. Detection process according to one of Claims 1-16, characterized in that at least one recognition

species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and a synthetic substance, a different natural substance or a different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

22. Detection process according to one of Claims 1-16, characterized in that a first recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid, a second recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and a synthetic substance, a natural substance or a natural substance derivative, preferably an antibody or antibody derivative.

23. Detection process according to one of Claims 1-16, characterized in that a first recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid, a second recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and another natural or unnatural, single-stranded or double-stranded nucleic acid, and the third recognition species is a further different natural or unnatural, single-stranded or double-stranded nucleic acid.

24. Detection process according to one of Claims 1-16, characterized in that a first recognition species is a synthetic substance, a natural substance or a natural substance derivative, preferably an antibody or antibody derivative, a second recognition species is a hybrid of a synthetic substance, a natural substance or a natural substance derivative, preferably an antibody or antibody derivative, and another natural substance or another natural substance derivative, preferably another antibody or antibody derivative, and a third recognition species is a further different synthetic substance, a natural substance or a natural substance derivative, preferably a further different antibody or antibody derivative.

25. Test system comprising at least two recognition species which recognize at least two different markers with formation of a complex.

26. Test system according to Claim 25, characterized in that at least one recognition species is immobilized on a support.

27. Test system according to Claim 25 or 26, characterized in that at least one recognition species is a natural or unnatural, single-stranded or double-stranded nucleic acid and at least one other recognition species is another natural or unnatural, single-stranded or double-stranded nucleic acid.

28. Test system according to Claim 25 or 26, characterized in that at least one recognition species is a synthetic substance, a different natural substance or a different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative, and at least one other recognition species is a synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

29. Test system according to Claim 25 or 26, characterized in that at least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and a synthetic substance, a different natural substance or a different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

30. Test system according to Claim 25 or 26, characterized in that at least one recognition species is a hybrid of a natural or unnatural, single-stranded or double-stranded nucleic acid and another natural or unnatural, single-stranded or double-stranded nucleic acid.

31. Test system according to Claim 25 or 26, characterized in that at least one recognition species is a hybrid of a synthetic substance, different natural substance or different natural substance derivative

other than a nucleic acid, preferably an antibody or antibody derivative, and another synthetic substance, different natural substance or different natural substance derivative other than a nucleic acid, preferably an antibody or antibody derivative.

32. Process for the production of a test system according to one of Claims 25-31, characterized in that the individual recognition species are assembled.

33. Process according to Claim 32, characterized in that at least one recognition species is immobilized on a support.

34. Use of the test system according to one of Claims 25-31 for the detection of the presence and/or absence of at least two different markers in a sample.

35. Use of the test system according to Claim 32 in the form of a diagnostic or in the form of an analyte.

36. Use of the test system according to Claim 32 or 33 for the detection of a disorder or for environmental analysis, in particular for the detection of disease pathogens, markers of diseases, toxins and/or allergens.

Fig. 1

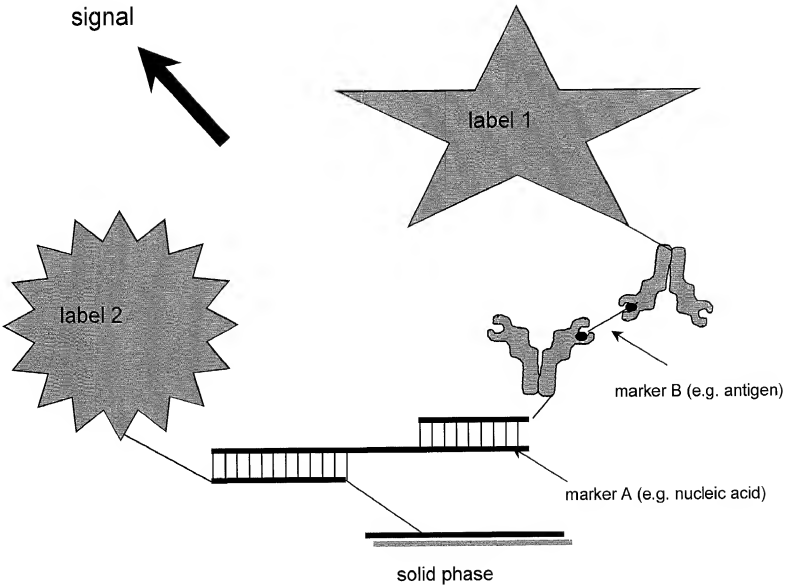


Fig. 2

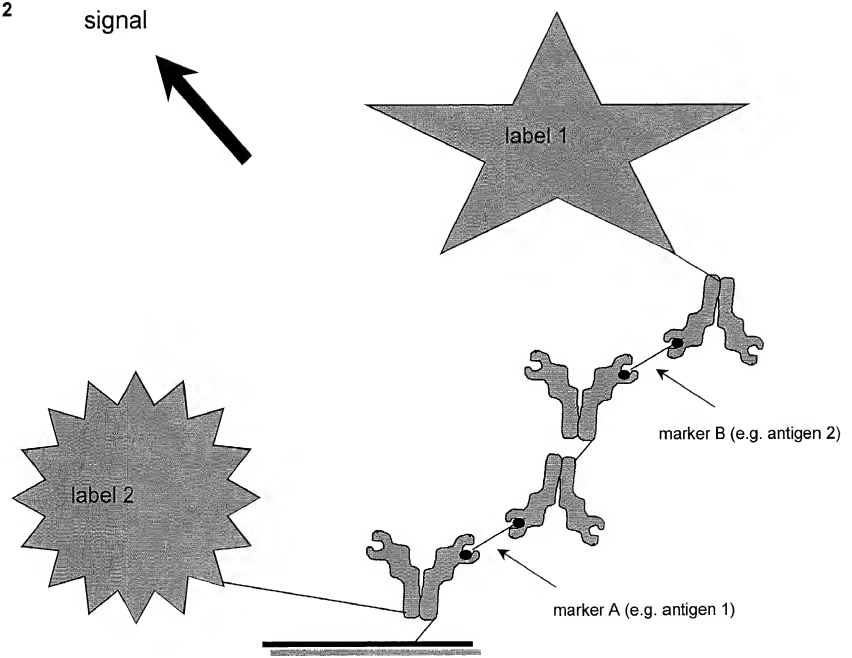


Fig. 3

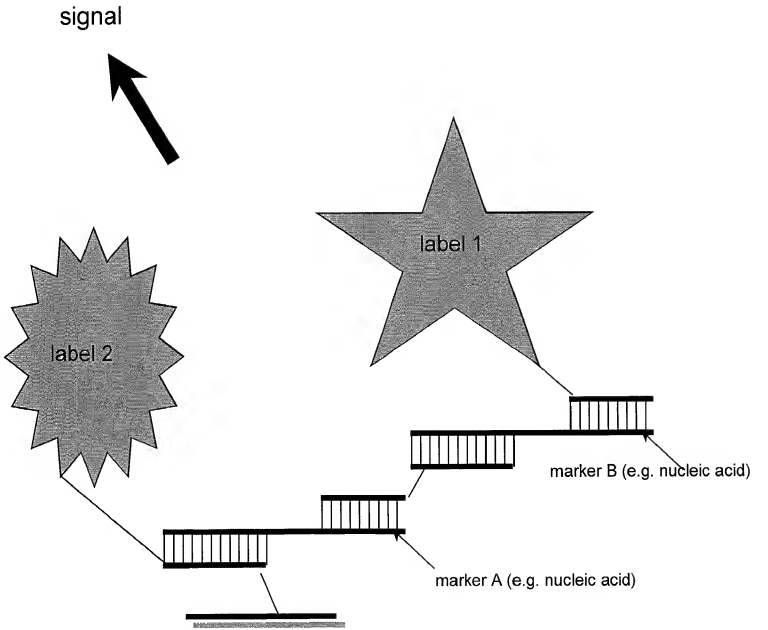


Fig. 4

example 1

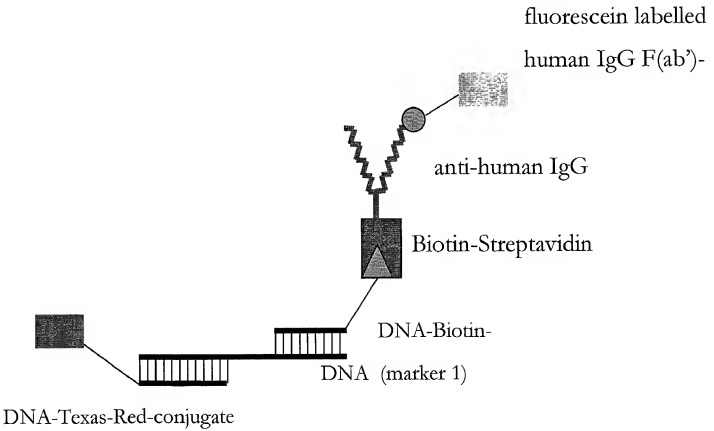
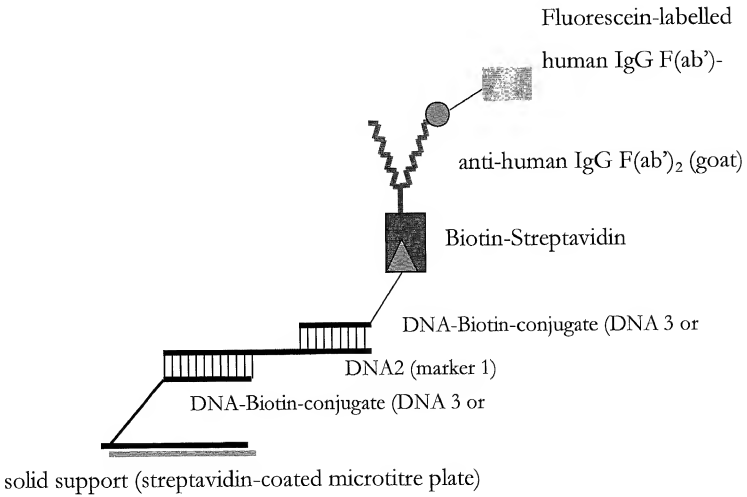


Fig. 5

example 2



PATENT
514485-3880

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY
(Includes reference to PCT International Applications)

FROMMER LAWRENCE & HAUG, LLP
File No.: 514485-3880

As a below named inventor, we hereby declare that:

Our residences, post office addresses and citizenships are as stated below next to our names.

We believe we are original, first and joint inventors (if plural, names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention ENTITLED:

TEST SYSTEM FOR DETECTING DIFFERENT MARKERS, AND PRODUCTION AND USE THEREOF

the specification of which:

is attached hereto

X was filed on June 21, 2001 as:

X United States Application Serial No. 09/868,824

X Corresponding to International Appln. No. PCT/EP99/10333 filed December 22, 1999 with amendments through DATE EVEN HEREWITH (if applicable, give details).

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to our to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

We hereby claim foreign priority benefits under Title 35, United States Code § 119 (a) - (d) or § 365 (b) of any foreign application(s) for patent or inventor's certificate, or § 365 (a) of any PCT International application(s) designating at least one country other than the United State of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT International applications designating at least one country other than the United States of America filed by us on the same subject matter having a filing date before that of the application(s) on which priority is claimed:
Prior Foreign/PCT Application(s) [list additional applications on separate page]:

Country (or PCT)	Application Number:	Filed (Day/Month/Year)	Priority Claimed: Yes	No
Germany	DE 19859912.9	December 23, 1998	x	

We hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

We hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) or § 365 (c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior United States or PCT International application(s) in the manner provided by the first paragraph of Title 35, United States Code § 112, we acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to us to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Prior U.S. (or U.S.-designating PCT) Application(s) [list additional applications on separate page]:

U.S. Serial No.:	Filed (Day/Month/Year)	PCT Application No.	Status (patented, pending, abandoned)

We hereby appoint William F. Lawrence, Registration No. 28,029, and FROMMER LAWRENCE & HAUG, LLP or their duly appointed associates, our attorneys or agents, with full power of substitution and revocation, to prosecute this application, to

make alterations and amendments therein, to file continuation and divisional applications thereof, to receive the Patent, and to transact all business in the Patent and Trademark Office and in the Courts in connection therewith, and to insert the Serial Number of the application in the space provided above, and specify that all communications about the application are to be directed to the following correspondence address:

William F. Lawrence, Esq.
c/o FROMMER LAWRENCE &
HAUG, LLP
745 Fifth Avenue
New York, NY 10151
FAX (212) 588-0500

Direct all telephone calls to: (212) 588-0800
to the attention of:
William F. Lawrence

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

INVENTOR(S):

Signature: _____ Date: 09.08.01

Full name of first or sole inventor: Thomas Wagner

Residence: ~~Römerstrasse 18, D-65719 Hofheim, Germany~~ Sonnenbühlstrasse 73, 78464 Konstanz, Germany DEX

Citizenship: German

Signature: _____ Date: 3.8.01

Full name of second inventor: Norbert Windhab

Residence: ~~Akazienstrasse 28, D-65075 Hattersheim, Germany~~ Hahneckstrasse 36, 65719 Hofheim, Germany DEX

Citizenship: Germany

Post Office Address(es) of inventors [if different from residence]:

NOTE: In order to qualify for reduced fees available to Small Entities, each inventor and any other individual or entity having rights to the invention must also sign an appropriate separate "Verified Statement (Declaration) Claiming [or Supporting a Claim by Another for] Small Entity Status" form [e.g. for Independent Inventor, Small Business Concern, Nonprofit Organization, Individual Non-Inventor].

SEQUENCE LISTING

<110> Wagner, et al.

<120> TEST SYSTEM FOR DETECTING DIFFERENT MARKERS, AND PRODUCTION AND USE THEREOF

<130> 514485-3880

<140> 09/868,824
<141> 2001-06-21

<150> PCT/EP99/10333
<151> 1999-12-22

<150> DE 198 59 912.9
<151> 1998-12-23

<160> 4

<170> PatentIn version 3.0

<210> 1
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Texas-Red labelled Oligonucleotide

<400> 1
aaatgcatgt cgtcgtgatg taaa 24

<210> 2
<211> 57
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide (DNA Marker)

<400> 2
attgtcataa tcatcttgag acgctttttt ttttttacat cacgacgaca tgcattt 57

<210> 3
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Biotin labelled Oligonucleotide

<400> 3
gcgtctcaac atgattatga caat 24

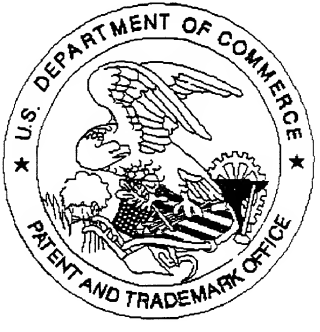
<210> 4
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Biotin labelled Oligonucleotide

<400> 4
aaatgcatgt cgtcgtgatg taaa 24

United States Patent & Trademark Office

Office of Initial Patent Examination -- Scanning Division



Application deficiencies found during scanning:

☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☐ Page(s)_____ of _____ were not present for scanning. (Document title)

☐ ***Scanned copy is best available.*** Drawing all Fig are Dark